

## PLASTICITY OF SPINY STELLATE CELLS IN CAT VISUAL CORTEX OF NORMAL AND VISUALLY DEPRIVED ANIMALS

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### ABSTRACT

We studied the relationships between the system of ocular dominance columns and the dendritic morphology of cells in layer 4 in cat visual cortex of normal and visually deprived animals. Geniculocortical axons from the ipsilateral eye were anterogradely labeled *in vivo* by injecting the fluorescent tracer Dil into layer A of the lateral geniculate nucleus. Subsequent intracellular staining in slice preparations allowed us to visualize simultaneously the dendritic arborizations of individual cells and the termination pattern of thalamic afferents. Our results show that the dendritic fields of spiny stellate cells in normal animals are biased away from the neighboring column. The dendritic fields of pyramidal cells in layer 4 were not influenced by the presence of a columnar border. This specific effect for spiny stellate cells was more pronounced in strabismic animals, where the normal eye alignment is disrupted and the input from the two eyes becomes more asynchronous. Monocular deprivation weakened the influence of the segregated input on the dendritic fields. Spiny stellate cells in the columns of the open eye had the tendency to confine their dendrites to the same column. However, spiny stellate cells in the columns of the deprived eye exhibited the opposite effect, their dendrites had a bias towards the neighboring column of the open eye. These results demonstrate that the dendritic fields of cortical neurons are shaped by patterns of afferent input. Our observations further indicate that the effects of visual deprivation on geniculocortical axons are accompanied by structural changes of their cortical target cells.

Many studies on the developmental mechanisms underlying the formation of the cortical functional architecture have been focused on the system of ocular dominance columns. In primary visual cortex, the input from the lateral geniculate nucleus (LGN) terminates predominantly in layer 4, where the afferents serving one eye are segregated into patches. As a consequence, cells

in layer 4 respond more strongly to the stimulation of one eye than the other. Initially during development, however, the afferents representing the two eyes are completely intermixed. Ocular dominance patches then emerge gradually by the progressive segregation of geniculocortical axons, which in the cat begins about 2 weeks after birth and is complete around 10 weeks postnatally. During this 'critical' period, peripheral manipulations can cause ocular dominance columns to form abnormally. For instance, if one eye is closed, the patches of LGN afferents representing the open eye are much larger than the patches representing the closed eye. As a consequence, most cells in these monocularly deprived animals respond only to the non-deprived eye. On the

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other hand, if the animals are made strabismic, so that the optical axes of the two eyes deviate from each other and the coordinated activity from the two eyes is interrupted, the segregation of the geniculocortical afferents into eye-specific patches are more sharply delineated and most cells respond exclusively to the stimulation of one eye only (for reviews see refs. 13, 17, 18).

The influence of visual experience on the formation of ocular dominance columns has been studied in great detail at the level of geniculocortical afferents. Much less is known, however, about the consequences of the reorganization of the afferent terminals on their target cells in layer 4. Recently it has been shown that cells in layer 4C $\beta$  of monkey striate cortex have their dendrites preferentially confined to only one ocular dominance column and rarely cross into the neighboring column (7). This suggests that the afferent segregation during normal development has played a significant role in defining the structure of their target cells. It is not known, however, whether different forms of visual deprivations leading to changes in the organization of geniculocortical axons also affect the dendritic branching patterns of their recipient cells in layer 4. In this report we review our experiments in which we examined whether postsynaptic cells in cat visual cortex show a similar degree of plasticity as their presynaptic axons from the LGN. Parts of these results have been published in abstract form (8), and a full account of this study will appear elsewhere (Kossel *et al.*, in preparation).

We examined the relationship between ocular dominance columns and the dendritic morphology of cells in layer 4 in adult cats that had been normally reared, and in animals that had been monocularly deprived by lid suture at the age of 3–5 weeks or made strabismic by an unilateral section of the medial rectus eye muscle at the age of 2–3 weeks. To label the pattern of ocular dominance columns, the fluorescent tracer DiI was injected by pressure into layer A of the LGN. After a survival time of 2–4 weeks the animals were deeply anesthetized and perfused with paraformaldehyde. Small blocks from area 17 were then prepared and cut in 300  $\mu$ m thick slices. Slices containing anterogradely labeled afferents were used for subsequent intracellular injections with Lucifer Yellow. This allowed to directly relate the dendritic branching patterns of cells in

layer 4 to the system of ocular dominance columns.

For a quantitative analysis of the structure of the dendritic fields concentric circles (spacing 25  $\mu$ m) around the cell body and a line through the cell body parallel to the border between the columns were drawn. This line divided the dendritic tree into two hemi-fields, one facing towards the border and one facing away from the border between the ocular dominance columns. The number of all intersections of the dendritic branches with the concentric circles in the two hemi-fields was then compared to determine quantitatively if a cell has more dendrites oriented away from the border or whether the cell's dendritic tree is symmetrical and not influenced by the presence of the columnar border. The quantitative analysis and a detailed description of the reconstruction of the anterograde labeling and the cell morphology will be presented elsewhere (Kossel *et al.*, in preparation).

A representative example of spiny stellate cells in a normal animal near to the border between two ocular dominance columns is illustrated in Fig. 1A. The cell has an asymmetrical dendritic field, more dendrites are oriented away from the border than oriented towards the border. In addition, the dendrites directed towards the border were often shorter than those running parallel or opposite to the border. A quantitative analysis of the total population of spiny stellate cells revealed that cells close to columnar borders (distance less than 40  $\mu$ m) on the average had significantly less intersections in the half-field facing towards the border than in the half-field facing away from it. Spiny stellate cells further away from the border had symmetrical dendritic fields, the number of intersections in both dendritic half-fields was not significantly different from each other. Unlike spiny stellate cells, the basal dendrites of pyramidal cells freely crossed the border between ocular dominance columns. Thus, the influence of thalamic afferents on the shape of the dendritic fields is specific for the cell class: only spiny stellate cells, but not pyramidal cells, are influenced by afferent segregation.

In accordance with previous observations (6, 11, 14), we found that anterogradely labeled ocular dominance columns in strabismic animals are more sharply demarcated from each other than in normal animals. Spiny stellate cells near these columnar borders had pronounced asym-

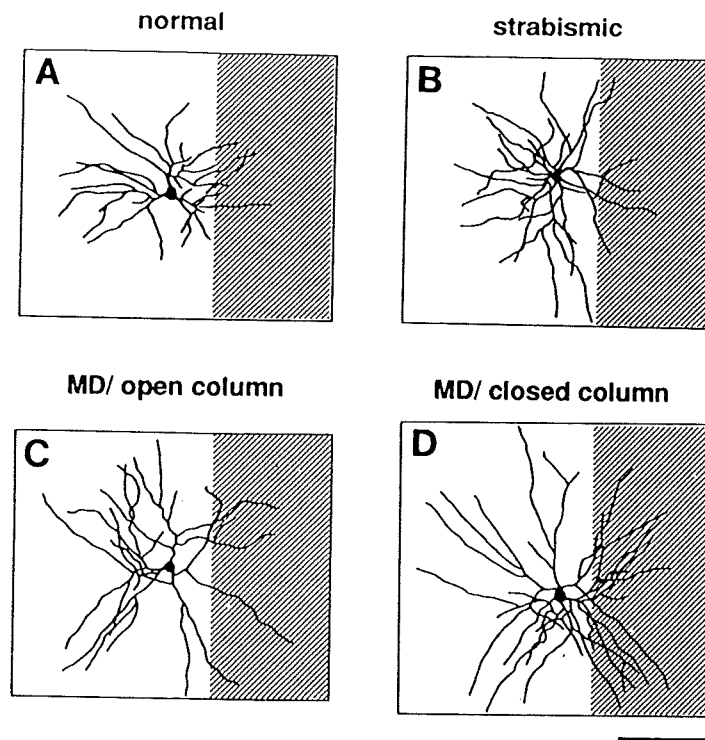


Fig. 1 Spiny stellate cells in normal and visually deprived cats near the border of an ocular dominance column (indicated by shading). A, normal animal; B, strabismic animal; C, monocularly deprived animal, the cell body is located in the column of the open (non-deprived) eye; D, monocularly deprived animal, the cell body is located in the column of the closed (deprived) eye. Scale bar: 100  $\mu$ m.

metric dendritic fields (Fig. 1B). The dendritic half-field facing towards the border contained fewer dendrites and was less dense than the half-field on the opposite side. Within 40  $\mu$ m from the border the dendrites of almost all spiny stellate cells were clearly biased away from the border. Cells more than 40  $\mu$ m away from the border had nearly symmetrical dendritic fields. The quantitative analysis revealed that the dendritic fields of spiny stellate cells in strabismic animals are more asymmetric near the columnar borders than spiny stellate cells in normal animals.

In monocularly deprived animals, cells are either located in the columns of the open eye or in the column of the closed eye. When analyzed separately, the behavior of dendritic fields of spiny stellate cells in the deprived and non-deprived columns was different. Some cells in the columns of the open eye were influenced by the

presence of a columnar border in the same way as cells in normal animals (Fig. 1C). Their dendritic fields were asymmetrical and biased away from the borders. Other cells, however, extended their dendrites freely into the column of the deprived eye. For the whole sample of spiny stellate cells in the columns of the open eye the number of intersections in the two dendritic half-fields was statistically not different. In contrast, none of the cells in the columns of the deprived avoided the neighboring column of the open eye. In the vicinity of the border (distance < 40  $\mu$ m) we rather observed the opposite effect: almost all cells had a bias towards the column of the open eye, with more dendrites running towards than away from the borders (Fig. 1D).

Our results show that the segregation of geniculocortical afferents into eye-specific patches in normal and strabismic animals is reflected in the shape of the dendritic field of their post-

synaptic target cells. The current hypothesis is that the segregation of geniculocortical afferents representing the two eyes is driven by neuronal activity. Physiological studies revealed that the relative timing between pre- and postsynaptic activity modifies the strength of cortical synapses: synchronous activity between pre- and postsynaptic neurons enhances, asynchronous activity weakens synaptic transmission (3, 4, 9, 12, 16). It has been proposed that these changes in synaptic efficacy lead to a remodeling of the terminal arborizations of geniculocortical axons. Axon collaterals are eliminated from cortical regions dominated by the other eye and they grow preferentially in regions dominated by the same eye (2, 10). Our observations suggest that a similar remodeling might occur at the level of the postsynaptic dendrites. Spiny stellate cells in layer 4 close to a columnar border extend their dendrites preferentially in the column where their cell body is located, and they appear to retract their dendrites from the neighboring column. Thus differences in the relative timing of activity between the two eyes lead to a segregation into eye-specific patches of both, presynaptic axons and postsynaptic dendrites. This hypothesis is supported by the results obtained in strabismic animals, where the input from the two eyes is more asynchronous than in normal animals. Strabismus causes a sharper segregation of geniculocortical axons into eye-specific patches and, as shown here, the bias of the dendrites of spiny stellate cells away from the termination zone of the afferents from the other eye is more pronounced than in normal animals.

The monocular deprivation experiments indicate that the organization of geniculocortical afferents within layer 4 is not only influenced by the relative timing, but also by the relative activity levels of the signals coming from the two eyes. The shrinkage of the cortical territories devoted to the deprived eye and the expansion of the territories devoted to the non-deprived eye which is seen after injections of transneuronal tracers into one eye was explained by an activity-dependent competition between the LGN afferents representing the two eyes (for review see refs. 17, 18). Recent studies based on intracellular labeling of individual geniculocortical axons indicated that the terminal arborizations representing the deprived eye are smaller, less dense, and have smaller synaptic boutons than

those representing the non-deprived eye (1, 5). These anatomical observations might explain the results from physiological experiments which indicated that LGN afferents serving the deprived eye, compared to the non-deprived eye, are less effective in driving their cortical target cells. Thus, even though the dendrites of some cells in the columns of the open eye cross freely into the column of the deprived eye, they receive only a weak synaptic input from this eye. The response of these cells is therefore clearly dominated by the input from the non-deprived eye. The situation is different for cells situated in the columns of the deprived eye, but close to a columnar border. These cells receive input from afferents serving the deprived eye. However, since their dendritic fields are biased towards the neighboring column, they also receive a significant input from geniculocortical arbors of the non-deprived eye. Because of the reduced efficacy in synaptic transmission of the afferents serving the deprived eye, the responses of these cells are therefore probably dominated by the input from the non-deprived eye. Thus, in monocularly deprived animals, ocular dominance columns visualized by anterograde labeling of geniculocortical afferents might not coincide with the physiologically defined grouping of cells according to eye preference. The extent of cortical regions dominated by the input from the deprived eye is smaller than the anatomical distribution of geniculocortical afferents serving the deprived eye. In fact, in studies which have combined anterograde tracing with electrophysiological recordings it was often found that when the electrode entered into the columns labeled from the deprived eye, the cells were still dominated by the input from the non-deprived eye (15).

In conclusion, we found that the segregation of geniculocortical afferents serving each eye during development plays an important role in defining the structure of the dendritic fields of spiny stellate cells. Perturbations of afferent segregation by manipulations of the visual input also caused alterations in the structure of the dendritic fields of cortical cells which might affect the functional properties of these neurons. Thus early experience not only influences the shape of the presynaptic geniculocortical arbors in layer 4, but also the dendritic fields of the postsynaptic spiny stellate cells.

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